

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/23378>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

Yearly Stepwise Increments of the Growth Hormone Dose Results in a Better Growth Response after Four Years in Girls with Turner Syndrome*

ARNE VAN TEUNENBROEK, SABINE M. P. F. DE MUINCK KEIZER-SCHRAMA, THEO STIJNEN, MAARTEN JANSEN, BARTO J. OTTEN, HENRIËTTE A. DELEMARRE-VAN DE WAAL, TOM VULSMA, JAN MAARTEN WIT, CATHARINUS W. ROUWÉ, H. MAARTEN REESER, JOS J. GOSEN, CISKA RONGEN-WESTERLAKEN, AND STENVERT L. S. DROP

(DUTCH WORKING GROUP ON GROWTH HORMONE)

Department of Pediatrics, subdivision of Endocrinology (A.T., S.M.P.F.M.K.-S., S.L.S.D.), Sophia Children's Hospital, Rotterdam; Department of Epidemiology and Biostatistics (T.S.), Erasmus University Rotterdam; Departments of Pediatrics, Wilhelmina Children's Hospital (M.J.), Utrecht; Sint Radboud University Hospital (B.J.O.), Nijmegen; Free University Hospital (H.A.D.-W.), Amsterdam; Academic Medical Center (T.V.), Amsterdam; Leiden University Hospital (J.M.W.), Beatrix Children's Hospital (C.W.R.), Groningen; Juliana Children's Hospital (H.M.R.), The Hague; Rijnland Hospital (J.J.G.), Leiderdorp; Sint Canisius-Wilhelmina Hospital (C.R.-W.), Nijmegen, the Netherlands

ABSTRACT

To optimize the growth promoting effect of growth hormone (GH), 65 previously untreated girls with Turner syndrome (TS), chronological age (CA) 2–11 yr, were randomized into 3 dosage regimen groups: A, B, and C, with a daily recombinant-human GH dose during 4 study years of 4-4-4-4, 4-6-6-6, and 4-6-8-8 IU/m² b.s.

The first GH dosage increase in groups B and C resulted in a significantly higher mean height velocity (HV) compared with constant dose group A. During the third year, when the dose was raised again only in group C, mean HV was significantly higher in groups B and C than in group A, and in group C compared with group B. In year 4 only group C mean HV remained significantly higher than group A. The pattern of change in HSDS_{CA} (Dutch-Swedish-Danish Turner references) was identical; however, in year 4 mean Δ HSDS_{CA} in group B also remained significantly higher than group A. After 4 yr GH treatment, the following was determined. 1) The mean Δ HSDS_{CA} was significantly higher for groups B and C compared with group A, but not significantly different between groups B and C. 2) Although significantly higher compared with estimated values for untreated Dutch girls with TS, bone maturation of the GH treated girls was not significantly different between groups. 3) It was positively related with the degree of bone age (BA) retardation at start of study and negatively with baseline CA. 4) Both the modified Index of Potential

Height (mIPH_{RUS}) and a recently developed Turner-specific final height (FH) prediction method (PTS_{RUS}), based on regression coefficients for H, CA, and bone age, showed significant increases in mean FH prediction, without significant differences between groups. PTS_{RUS} values were markedly higher than the mIPH_{RUS} values.

Dose dependency could be shown for the area under the curve (AUC) for GH, but Δ HSDS_{CA} was not linearly related with AUC. Baseline GH binding protein (BP) levels were in 84% of the cases within the normal age range; the decrease in mean levels after 6 months GH was not significant. Mean insulin-like growth factor I (IGF-I) and IGFBP-3 plasma levels increased significantly, without significant differences between groups. Δ HSDS_{CA} during GH was dependent on IGF-I plasma levels at baseline and during the study period, β -0.002 and β -0.0004. Thus, a stepwise GH-dosing approach reduced the "waning" effect of the growth response after 4 yr treatment without undue bone maturation. FH prediction was not significantly different between treatment groups. Irrespective of the GH dose used, initiation of GH treatment at a younger age was beneficial after 4 yr GH when expressed as actual cm gained or as gain in FH prediction, but was not statistically significant when expressed as Δ HSDS_{CA} over the study period. (*J Clin Endocrinol Metab* 81: 4013–4021, 1996)

STUNTED growth is an almost invariable hallmark of girls with Turner syndrome (TS). Although these girls are not clearly GH-deficient (GHD) (1–4), GH therapy results in

a marked increase of height velocity (HV) (5–7). Studies in GHD patients (8–10) and in TS (5–7, 11–13) have shown that the growth response to GH treatment is dependent on the dose and frequency of administration. Frasier *et al.* (14) reported in a 1 yr, parallel study in prepubertal GHD children that doubling the GH dose of 30 mU/kg thrice weekly im resulted only in a 1.3-fold increase of the first year HV. Also in TS, daily GH injections were more effective than the same weekly dosage in two or three weekly injections (12). In the past years, the most commonly used GH dosages in TS varied from 2–4 IU/m² body surface/day sc (5–7, 12, 15), where 4 IU/m² is equivalent to 0.045 mg/kg. In studies using GH dosages only, up to 4 IU/m²/day, the growth rate outweighed the accelerated bone maturation (13, 16), and thus,

Received February 14, 1996. Revision received May 30, 1996. Accepted June 3, 1996.

Address correspondence and requests for reprints to: A. van Teunenbroek, M.D., subdivision of Endocrinology, Sophia Children's Hospital, Dr Molewaterplein 60, 3015 GJ Rotterdam, the Netherlands.

* This study was supported by Novo Nordisk A/S, Denmark. Interim analyses have been presented at the Fourth Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology (ESPE) in San Francisco, June 1993, and at the 33rd Annual Meeting of the ESPE in Maastricht, June 1994. A preliminary four-year analysis has been presented at the ESPE in Edinburgh, June 1995.

final height (FH) prediction improved more than with lower GH doses (5, 7, 12, 15). An earlier GH treatment study of girls with TS in the Netherlands (16) showed a doubling of the HV in the first year of treatment with 4 IU GH/m²/day compared with pretreatment values. However, this increase could not be maintained during the subsequent years of treatment. This so-called "waning" effect has also been reported by others (10, 17). In GHD patients, a similar effect is observed, which can be overcome by a 2- to 3-fold increase of the GH dose (10, 18).

Furthermore, studies in GHD patients have demonstrated the importance of early diagnosis and therapy. GH treatment prevented further loss of stature but could not make up the deficit at diagnosis (19). The previous Dutch studies of Turner syndrome confirmed that the growth response in younger girls was better than in older girls (12, 13, 16). In contrast to the logarithmic relationship between GH dose, given thrice weekly im, and HV in GHD children, the effect of GH on insulin-like growth factor (IGF)-I plasma levels has been shown on a linear scale between 0 and 3 IU GH/m²/day in GHD adults (20). In the present study of TS, this concept is extended to the 4–8 IU/m²/day GH range.

To optimize GH treatment in TS, we investigated whether 1) a yearly stepwise increment of the GH dose could maintain or augment the initial increase in HV and thereby improve FH prediction. In addition, we investigated whether 2) treatment from a young age onwards could improve FH prediction. Moreover, in a subgroup of 12 girls the GH-IGF-I axis was studied in detail under GH treatment.

Subjects and Methods

Study group

Sixty-eight previously untreated girls with TS were enrolled in a 4-yr, multicenter GH dose-response study. The diagnosis was confirmed by lymphocyte chromosomal analysis. Clinical data and karyotype of the girls are listed in Table 1. Inclusion criteria were: a chronological age (CA) between 2 and 11 yr, height below the 50th percentile for Dutch children (21), and a normal thyroid function. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure caused

by other disorders or emotional deprivation, hydrocephalus, previous use of drugs that could interfere with GH therapy, and Tanner puberty stage of at least B2 (22). No provision was made with regard to the baseline GH stimulation tests.

Study design

The girls were randomized into three GH dosing groups with stratification according to CA and height standard deviation score (HSDS_{CA}):

- (n = 23) 4 IU/m² body surface (equivalent to 0.045 mg/kg)/day for 4 yr,
- (n = 23) 4 IU/m² in the first year, followed by 6 IU/m²/day during the second through fourth yr,
- (n = 22) 4 IU/m² in the first year, 6 IU/m² in the second year, and 8 IU/m²/day during the third and fourth yr.

Biosynthetic (B)-hGH (Norditropin, Novo Nordisk A/S, Denmark) was given sc at bedtime by means of a pen injection system (Nordject 24). None of the girls received estrogens during the 4-yr study period. Written, informed consent was obtained from the parents or custodians of each child. The study protocol was approved by the Ethics Committee of each participating center.

Growth evaluation

Height measurements were determined at baseline and three times per month by one investigator (A.T.) according to the methods of Cameron (23), using a Harpenden stadiometer. Height was expressed as sd-score for CA (HSDS_{CA}, HVSDS_{CA}) using the Dutch-Swedish-Danish (DSD) Turner data (24) or reference data of normal Dutch (21) girls. The gain in height for untreated girls with TS was estimated from the equations of the DSD Turner data (24), in which the height of an average girl with TS is indicated by her CA. Midparental height (MPH) was adapted for Dutch reference data (21) with the addition of 3 cm for secular trend: MPH = 1/2 × (H_{mother} + H_{father} - 12) + 3 cm. The degree of obesity was expressed as body mass index (BMI) sd score (25). Bone age (BA) was determined by one investigator (A.T.) according to Tanner & Whitehouse radius, ulna, short-bones (RUS) score (26). Bone maturation (Δ BA/ Δ CA) was compared with estimated values from the equations of untreated Dutch Turner girls (27); in these equations the BA of an average girl with TS is indicated by her CA. FH prediction was estimated using the modified Index of Potential Height (mIPH_{RUS}) method (28, 29) and a recently developed Turner-specific method (PTS_{RUS}) (29). Both methods comprise Dutch Turner references. Analogous to the Tanner and Whitehouse mark 2 FH prediction method for normal children, the PTS method gives smoothed regression coefficients for H, CA, and BA.

Biochemical parameters

At baseline all girls underwent a GH provocation test. Arginine 0.5 g/kg body weight was infused in 30 min. Blood samples were drawn at 15-min intervals from -15 to +60 min and every 30 min during the second hour.

At baseline, 6 months after initiation of GH, and 6 months after each GH dosage increase, a 24-h GH profile was performed in a subgroup of 12 girls of group C. Starting at 0830 in the morning, blood was withdrawn from an indwelling venous catheter with a heparin lock. Blood was collected every 20 min for GH measurement, and at the start a single additional sample was obtained for measurement of IGF-I and IGFBP-3. The girls kept normal diets served at hospital mealtimes and kept normal activity and sleeping habits. GH was injected before going to bed. At the above study time points blood was collected from all girls for the determination of IGF-I and IGFBP-3, and at 42 and 48 months only for IGF-I. GH binding protein (GHBP) was determined at baseline and 6 months after initiation of GH therapy. All blood samples were stored on ice for no more than 3 h until centrifugation. The plasma samples were frozen (-20 °C) until assayed.

Hormone assays

The RIA measurements of plasma GH, IGF-I, and IGFBP-3 were performed as described previously (30–32). The 95th percentile for peak pubertal levels in a normal female population for IGF-I and IGFBP-3 are

TABLE 1. Baseline data for each treatment group

	Group A	Group B	Group C
Number of girls	22	22	21
CA (yr)	6.1 (2.1)	6.7 (2.4)	6.5 (2.4)
RUS BA (yr)	5.5 (2.2)	6.0 (2.5)	5.8 (2.4)
HSDS _{CA} (RvW)	-2.7 (0.9)	-2.4 (1.0)	-2.6 (1.0)
HSDS _{CA} (DSD)	0.06 (1.03)	0.42 (1.05)	0.18 (1.06)
HVSDS _{CA} (DSD)	0.32 (0.80)	0.08 (0.91)	0.16 (0.71)
mIPH _{RUS} (cm)	147.8 (7.7)	148.0 (4.8)	147.4 (5.5)
PTS _{RUS} (cm)	145.6 (5.9)	147.6 (5.2)	146.3 (5.0)
MPH (cm)	169.4 (5.9)	170.6 (6.0)	169.7 (5.7)
BMI-SD score	0.24 (1.20)	0.27 (1.33)	0.20 (1.29)
Karyotype: 45,X	18 (82%)	21 (96%)	16 (76%)
Karyotype: other	4 (18%)	1 (4%)	5 (24%)
maxGH (ATT) mU/L	23.5 (16.6)	20.4 (15.2)	25.6 (16.4)

Values are given as mean (SD). CA, chronological age; RUS BA, RUS-bone age; SDS standard deviation score; H, height; HV, height velocity; RvW, Dutch reference standard for girls; DSD, Dutch-Swedish-Danish Turner references; mIPH_{RUS}, modified Index of Potential Height; PTS_{RUS}, Turner-specific final height prediction using RUS bone age; MPH, midparental height; BMI, body mass index-SDs; maxGH (ATT), maximum GH plasma concentration after arginine stimulation.

700 µg/L and 5 mg/L, respectively. Plasma GH binding protein (GHBP) was determined by ligand-mediated immunofunctional assay (LIFA) (33, 34). All measurements were performed in the same laboratories.

Statistical analyses

Results are expressed as mean (SD), unless indicated otherwise. Differences between groups were tested by Student's *t*-tests or by a oneway ANOVA (followed by the Student-Newman-Keuls test for multiple comparisons between groups at the $P = 0.05$ level). Differences between points in time were tested by paired Student's *t*-tests. The Kruskal-Wallis test was used to test for differences between stimulated maximum GH levels and Tanner breast-stage groups, the Chi-square test for differences between karyotype groups (45, X, and others). Correlations were tested with Pearson's linear correlation coefficient. For this purpose, IGF-I and IGFBP-3 plasma levels were transformed into log-values. To study the relation between growth response variables (the change in HSDS_{CA} , HVSDS , bone maturation, or PTS_{RUS}) and growth parameters measured at baseline [CA, BA, BA retardation (= CA-BA), HV, maximal GH peak after stimulation, SD scores for BMI, H, and HV], adjusted for the dose regimen (*i.e.* group), multiple linear regression (MLR) analyses were used. Statistical procedures were performed using the SPSS/PC+ program version 4.0 (SPSS Inc, Chicago, IL). A repeated measures ANOVA model (adjusted for dose-increment steps and duration of treatment) was used to determine the influence of baseline IGF-I levels on those during GH therapy and of IGF-I levels (at baseline and during GH therapy) on HSDS_{CA} during GH therapy, using BMPD module 5V. A P value of less than 0.05 was considered significant.

The spontaneous 24-h GH profiles at baseline were analyzed with the Pulsar program developed by Merriam and Wachter and described previously (30). The area under the curve (AUC) was determined after the GH injection at 1600 h using the trapezoidal rule. The total body clearance was calculated from the injected dose divided by the AUC. The time interval from GH injection to maximum GH levels was recorded as T_{max} . To determine the elimination half-time ($t_{1/2}$) a linear regression analysis was performed on the GH levels starting from 1 h after T_{max} .

Results

Clinical data

In each group only one girl dropped out of the study for the following reasons: noncompliance, alleged increase of muscle mass and decline in school performance, and desire to initiate estrogen therapy before the end of the study period. Eight girls changed during the course of the study from Tanner puberty stage B1 to B2, at a median age of 13.2 (range 10.9–15.0) yr. Their distribution among the treatment groups A, B, and C was 2, 4, and 2 girls, and among karyotypes (45, X and others) 4 and 4 girls, respectively. There were no significant differences between these girls and the girls without signs of endogenous estrogen production with respect to growth response and bone maturation after 4 yr GH therapy within each dose group. The number of adverse events was small, all were mild and transient.

Growth response

Compared with pretreatment, mean HV increased significantly for all three groups from about 6 cm/yr to 10 cm/yr in the first year of GH therapy. Thereafter, a waning of the growth response was observed (Fig. 1). In the second year mean HV in groups B and C on a 50% higher GH dose were significantly higher compared with group A. When subsequently, in group C only, the dose was increased once again, mean HV in groups B and C were both significantly higher than in group A, but in group C also significantly higher compared with group B. In the fourth year of GH treatment

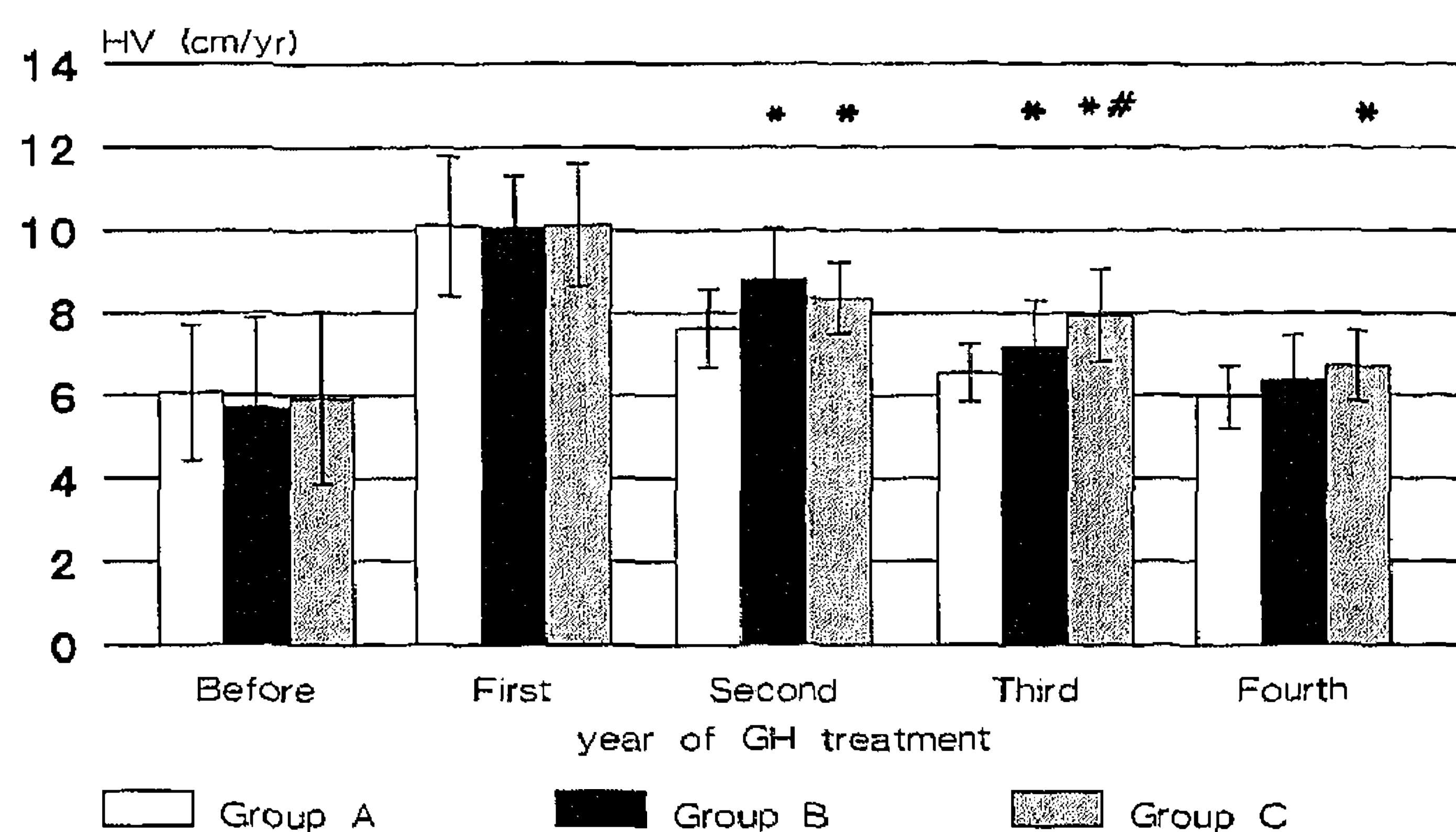


FIG. 1. Development of mean (SD) HV (cm/yr) for treatment groups A (□), B (■), and C (▨). Significant differences ($P < 0.05$) compared with group A (*) and with group B (#) are indicated.

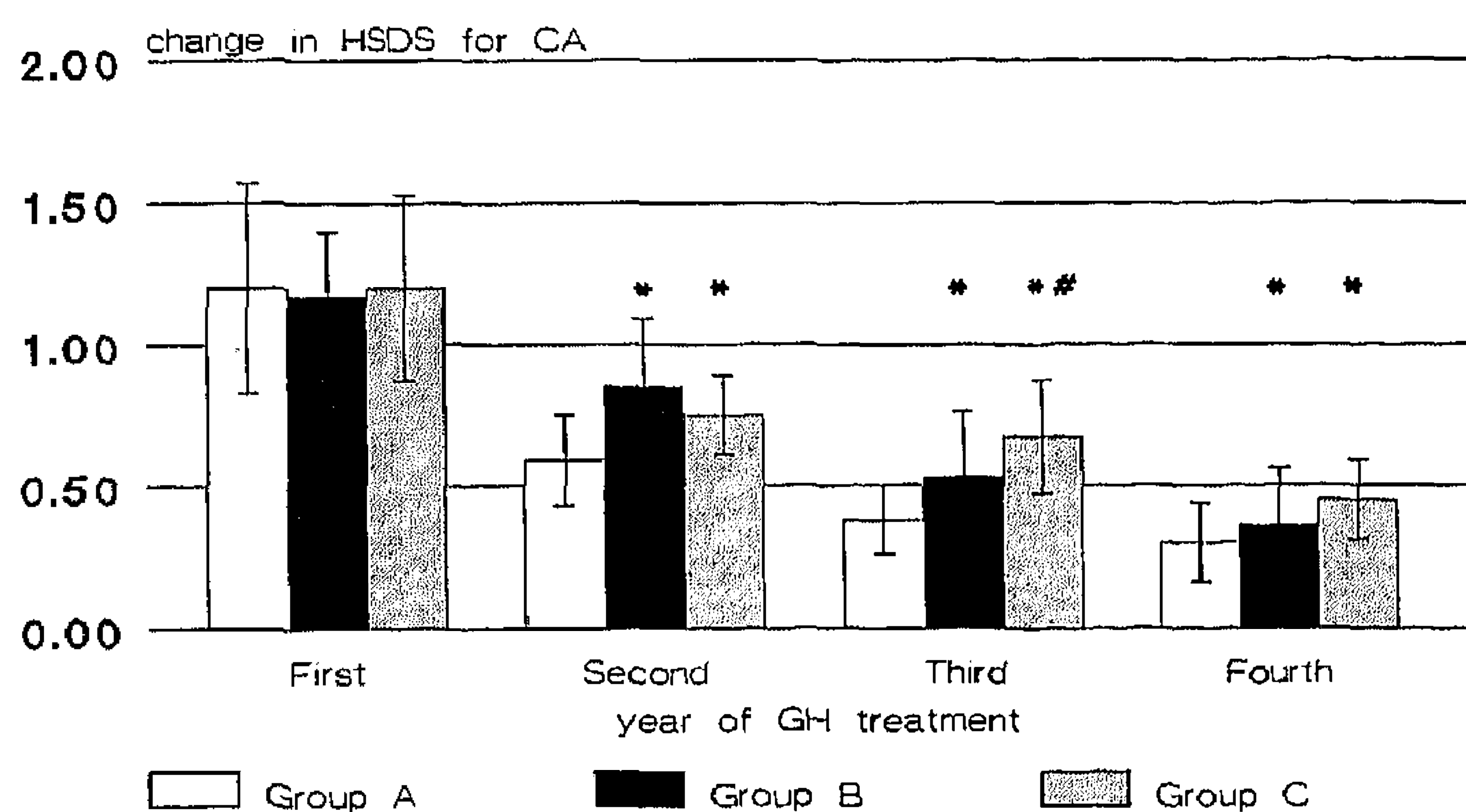


FIG. 2. Development of mean (SD) change in HSDS_{CA} for treatment groups A (□), B (■), and C (▨). Significant differences ($P < 0.05$) compared with group A (*) and with group B (#) are indicated.

only in group C the mean HV remained significantly higher than group A. During the first year of treatment 29% of all girls managed to double their HV.

If the growth response is represented as change in HVSDS_{CA} relative to prestudy values (see Table 1), $\Delta\text{HVSDS}_{\text{CA}}$ in groups B and C was significantly higher than in group A in the second through fourth year of GH therapy. However, in the third and fourth year ΔHVSDS in group C was not significantly different from group B.

After the first dose-increment for both groups B and C, the change in HSDS_{CA} from the first year was significantly higher for the combined groups B and C compared with group A ($P < 0.0001$). The second dose-increment in the third year of treatment, as well as in the combined third and fourth year, resulted in a significantly higher change from year 2 in HSDS_{CA} for group C compared with group B, P values 0.04 and 0.02. The increase in mean HSDS_{CA} was highest in the first year of GH (>1 SDs), without a difference between groups (Fig. 2). In the subsequent years of treatment, the change in mean HSDS_{CA} showed the same pattern as that of HV. The mean increment in HSDS_{CA} over 4 yr was significantly higher for groups B and C compared with group A. However, the gain was not significantly different between groups B and C (Table 2). The change in HSDS_{CA} after 4 yr was unrelated to karyotype. When the gain in height was corrected for the estimated gain for untreated girls, the re-

TABLE 2. Mean (SD) change during 4 years of GH treatment for every treatment regimen

	Group A	Group B	Group C	P
Δ HSDS _{CA} (DSD)	2.46 (0.53)	2.91 (0.54) ^a	3.07 (0.57) ^a	0.004
height gain (cm)	12.4 (2.8)	15.3 (3.1) ^a	15.7 (2.5) ^a	0.0004
HVSDS _{CA} (DSD) (y4-prestudy)	1.60 (0.97)	2.43 (1.22) ^a	2.62 (0.99) ^a	0.007
Δ BA/ Δ CA (y/4y) with GH	5.2 (0.7)	5.4 (0.9)	5.3 (1.0)	NS
Δ BA/ Δ CA (y/4y) untreated	4.0 (0.5)	3.8 (0.6)	3.9 (0.6)	NS
Δ mIPH _{RUS} (cm)	4.9 (4.8)	6.6 (3.1)	7.1 (4.5)	NS
Δ PTS _{RUS} (cm)	12.3 (3.8)	14.1 (3.1)	14.7 (3.5)	NS

P, level of significance in a oneway ANOVA; Δ , change during a period of time for a variable; height gain, gain in cm over estimated untreated values; HV, height velocity; SDS, standard deviation score; DSD, Dutch-Swedish-Danish Turner references; Δ BA/ Δ CA, bone maturation (RUS-score); change in BA during 4 years GH treatment; untreated values were estimated using Dutch Turner references; mIPH_{RUS}, modified Index of Potential Height; PTS_{RUS}, Turner-specific final height prediction using RUS bone age.

^a Significantly different from group A.

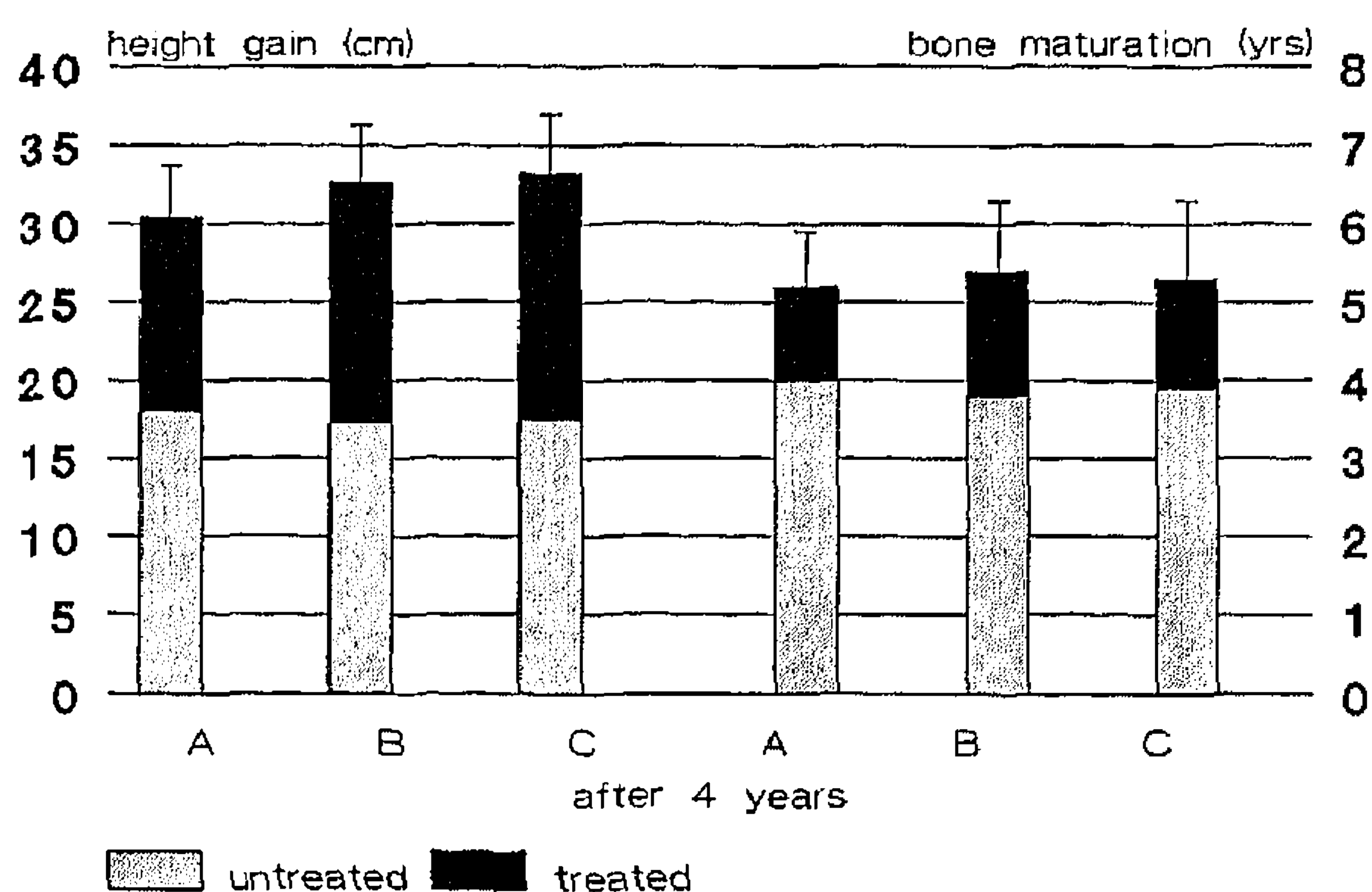


FIG. 3. Stacked mean (SD) of treated (■) over estimated untreated values (▨) in girls with TS after four yr GH treatment, both for gain in height (in cm) and for bone maturation (y/4y) in each of the treatment groups A, B, and C.

sults were similar (Table 2 and Fig. 3). The range of the gain in height over estimated untreated values for all girls was 5.5–21.9 cm; for four girls (all Group A), it was below 10 cm.

Bone maturation

The change in RUS BA over the change in CA was not significantly different between groups over 4 yr (Table 2), nor during any individual year of treatment. The mean values differed somewhat between the years: for all groups the highest advance was found during the third year and the lowest during the fourth year of GH (data not shown). Compared with estimated values for untreated TS girls, bone maturation of the GH treated girls was significantly higher in every year, except for group A in the second and fourth year; for 4-yr results see Fig. 3. There was no significant difference in the change in bone maturation over 4 yr between the girls with breast-stage B1 and B2 within groups. Bone maturation after 4 yr of treatment was positively related with the degree of BA retardation at start of study (β : 0.12) and negatively with baseline CA (β : -0.04).

Final height (FH) prediction

Mean FH prediction increased significantly for all groups after 4 yr GH treatment (Table 2); values with the PTS_{RUS} method were markedly higher compared with the mIPH_{RUS} method. Significant differences between groups for the 4 yr change in either FH prediction method were not found,

though mean values with both methods in groups B and C were higher than those in group A.

GH measurements

Baseline Arginine-stimulated GH plasma levels ranged from 3–74 mU/L (Table 1). The stimulated GH levels (mU/L) were subdivided in the following level-ranges: less than 10, at least 10 and less than 20, and at least 20, with 9, 28, 31 girls, respectively. These numbers were similarly distributed among the 3 treatment groups. The girls with maximum stimulated GH levels below 20 mU/L did not differ significantly from those with normal stimulated levels (>20 mU/L) in their growth response expressed as the change in HSDS_{CA} after 4 yr of GH treatment. Maximum stimulated GH levels were significantly negatively correlated with BMI-SD score at baseline ($r = -0.31$, $P = 0.01$). At baseline, the spontaneous and stimulated maximum GH levels in group C were not significantly different and were positively correlated ($r = 0.5$, $P = 0.05$).

Table 3 includes some of the calculated variables of the spontaneous 24-h GH profiles of 12 girls of Group C (at baseline). There was no correlation between any of these characteristics and prestudy HSDS_{CA}. Furthermore, the characteristics of the 24-h GH profile tests 6 months after each dose-increment are shown. There was a significant, dose-dependent increase of the maximum GH level and the AUC. In contrast, the T_{max} , the clearance, and the elimination half-life were not significantly different between the 3 GH doses. The latter is indicated by the parallelism between the curves after the maximum has been reached (Fig. 4).

GH binding protein (GHBP)

Baseline measurements showed no differences between groups (Table 4), the mean (SD) for all girls being 229.4 (127.1) pmol/L. Compared with girls in a normal population (34), 85% of the study group had GHBP levels within the normal age range, 9 girls (14%) had levels that were above normal, and only 1 girl (1%) had levels below normal. Baseline GHBP levels as well as the change from baseline after 6 months were not significantly different between the girls with stimulated GH levels above or below 20 mU/L. GHBP levels after 6 months treatment did not differ significantly from baseline.

TABLE 3. Median (range) values for characteristics of the 24-h GH profile tests for the 12 girls of Group C at baseline (determined by Pulsar) and 6 months after each dose-increment step (see *Methods* section)

	Baseline	4 IU/m ² /day	6 IU/m ² /day	8 IU/m ² /day
Number of peaks	10 (8;13)			
Mean GH (mU/L)	4.3 (1.64;6.78)			
Max GH (mU/L)	23.5 (8;42)	63 (27;127)	113 (57;174) ^a	197 (101;405) ^{a,b}
AUC (mU/L × 24 h)	99.1 (39.8;160.3)	341 (160;157)	570 (352;896) ^a	962 (554;1530) ^{a,b}
Clearance (mL/min)		420 (224;680)	412 (203;568)	362 (214;566)
t _{1/2} (hrs)		0.5 (0.2;0.7)	0.5 (0.3;0.7)	0.5 (0.2;0.9)
T _{max} (hrs)		2.9 (1.3;4.3)	3.2 (1.3;6.0)	3.2 (1.7;5.3)

No of peaks, number of peaks; mean GH, overall mean GH plasma concentration; max GH, maximum GH plasma concentration; AUC, area under the time-concentration curve; clearance, total body clearance; t_{1/2}, elimination half-time; T_{max}, time to peak value. Values in parentheses are high and low.

^a Significantly greater compared with 4 IU/m²/day at $P < 0.05$ level.

^b Significantly greater compared with 6 IU/m²/day at $P < 0.05$ level.

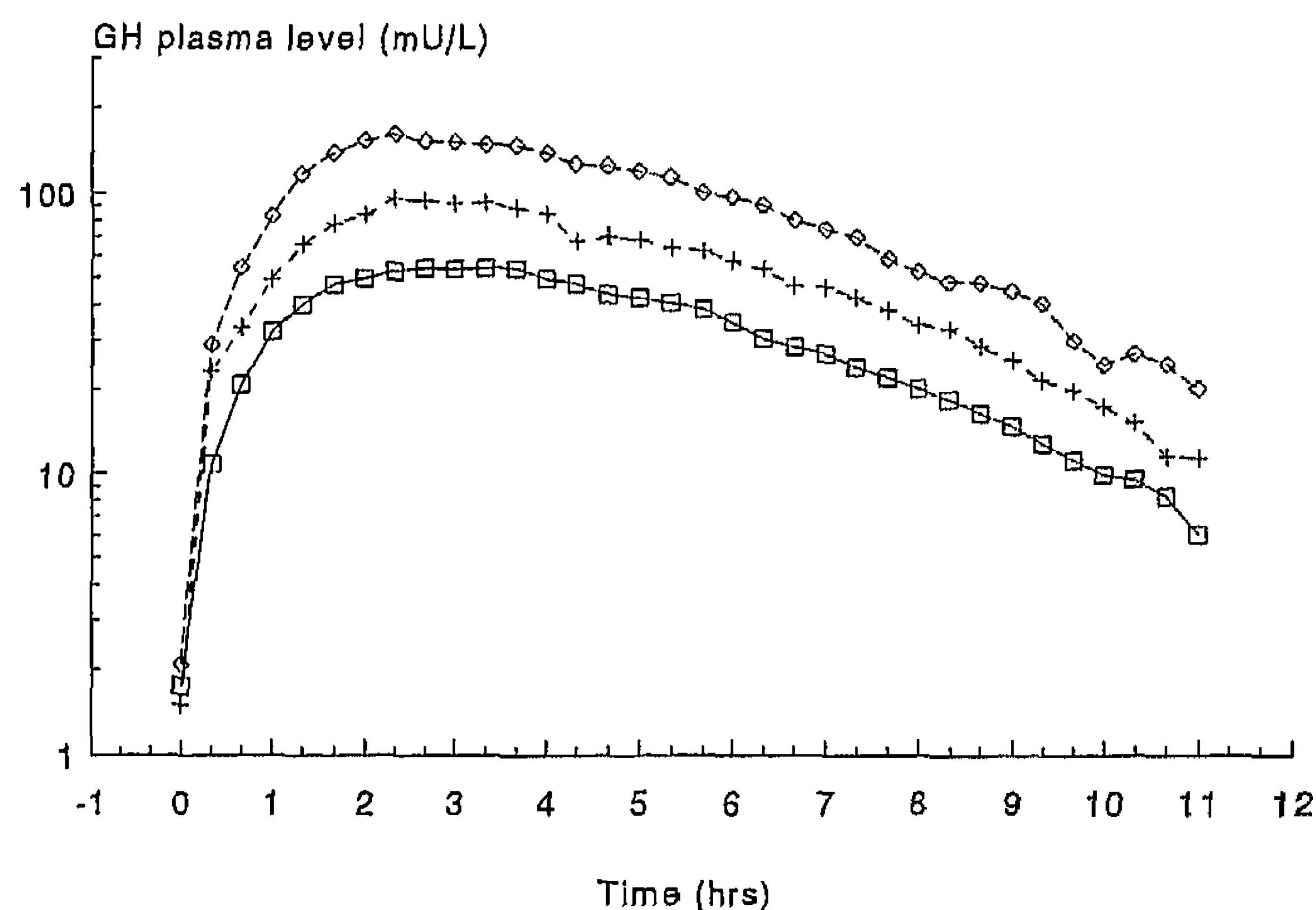


FIG. 4. Mean 24-h GH plasma curves (logarithmic scale) vs. time for each of the three GH dosages (4 IU —□—, 6 IU —+—, and 8 IU —◇—) employed in a subgroup of 12 girls of Group C, 6 months after initiation and following each GH dose-increment.

IGF-I and IGF binding protein-3 (IGFBP-3)

At each time-point large interindividual differences existed within groups (Table 4). Mean baseline IGF-I level of group B was higher compared with the other groups. Within groups, each point in time was significantly higher than the previous, except for 30 months (all groups) and 42 months (group B). Not until 30 months after the start of therapy did IGF-I levels (adjusted for baseline levels) for groups B and C become significantly higher compared with group A ($P < 0.004$), but at 48 months only group C was still significantly higher than group A ($P = 0.008$). The repeated measures model showed that the change in IGF-I levels during GH therapy was dependent on the dose, the duration of treatment, and baseline IGF-I level.

At baseline, mean IGFBP-3 levels for group B were higher compared with the other two groups. After adjustment for baseline, IGFBP-3 levels were not significantly different between groups (Table 4). Mean IGFBP-3 levels only increased significantly after 6 months of treatment ($P < 0.0001$). At the end of study 31% of the girls had plasma IGF-I levels and 35% had IGFBP-3 levels higher than the 95th percentile for normal girls at the pubertal peak. There were no differences between treatment groups.

The ratio of IGF-I and IGFBP-3 levels showed an increase over time, but there were no significant differences between groups. Log-values of IGF-I and IGFBP-3 levels both at baseline and their change after 30 months revealed a significant correlation ($r = 0.76$, $P < 0.0001$ and $r = 0.25$, $P = 0.04$, respectively).

Determinants of growth response

Multiple Linear Regression analyses showed that there were no significant relationships between 1) HVSDS_{CA} in the fourth year GH (dependent variable) and pretreatment HVSDS; 2) the change in HSDS_{CA} after 4 yr GH (dependent variable) and baseline: CA, BA RUS, BA retardation, HSDS_{CA}, or Arginine-stimulated maximum GH levels ($\beta = -0.008$; $P = 0.07$); and 3) prestudy HV or HVSDS and baseline IGF-I, or IGFBP-3 levels, or between the IGF-I to IGFBP-3 ratio. However, the 4-year change in HSDS_{CA} was significant, negatively related to baseline IGF-I and IGFBP-3 levels and their ratio (β -values -0.006 , -0.32 , and -0.015 , respectively); even when the baseline IGF-I and IGFBP-3 concentrations were expressed as SD score relative to CA only for the girls with a baseline CA below 10 yr ($n = 64$). The change in HSDS_{CA} after 30 or 48 months GH treatment was also significant, positively related to the change in IGF-I, and IGFBP-3 levels after 30 months of GH treatment, but not to their ratio. The repeated measures model with dose-increment steps and duration of treatment as covariates also showed that the change in HSDS_{CA} during GH therapy was dependent on IGF-I plasma levels at baseline and during the study period ($\beta -0.002$ and $\beta 0.0004$). The gain in height over estimated untreated values at the end of the study (dependent variable) was significantly negatively correlated ($P < 0.0001$) with age at start of treatment. The change in PTS_{RUS} after 4 yr GH (dependent variable) was significantly negatively related with CA or BA retardation at the start of the study, as well as with bone maturation during the study period. Finally, there was no linear relationship between 1) the change in HSDS_{CA} and in the plasma GH AUC at each corresponding point in time; 2) the change in IGF-I or IGFBP-3 plasma levels, or in the IGF-I to IGFBP-3 ratio and the change in AUC at each corresponding point in time; and 3) baseline GHBP levels and baseline CA, HSDS_{CA}, HV, stimulated GH levels. Only GHBP levels and BMI-SDS at the start of treatment were related ($r = 0.45$, $P = 0.003$).

TABLE 4. Mean (SD) of IGF-I, IGFBP-3, and GHBP levels for every treatment regimen at baseline, 6 months after initiation of GH therapy, and each GH dose-increment, and at 42 and 48 months of GH treatment

	Gr	Baseline	6 months	18 months	30 months	42 months	48 months
IGF-I (mcg/L)	A	76.5 (32.7)	213.9 (97.5)	276.6 (91.2)	371.2 (142.8)	393.1 (124.8)	512.7 (143.7)
	B	104.9 (41.2) ^a	263.7 (126.4)	363.6 (169.2)	562.3 (227.6) ^b	525.3 (168.6) ^b	608.1 (191.6)
	C	85.8 (37.7)	245.1 (100.5)	348.0 (170.7)	501.8 (139.7) ^b	526.5 (154.5) ^b	678.6 (179.0) ^b
IGFBP-3 (mg/L)	A	2.54 (0.57)	4.15 (0.79)	4.22 (0.86)	4.08 (0.92)		
	B	2.99 (0.75)	4.71 (1.34)	5.08 (1.26)	4.99 (1.44)		
	C	2.78 (0.55)	4.29 (0.90)	4.58 (0.97)	4.62 (0.80)		
GHBP (pmol/L)	A	217.1 (94.0)	201.0 (81.6)				
	B	256.9 (149.4)	219.8 (95.5)				
	C	224.1 (140.4)	223.4 (164.8)				

Gr, group.

^a Significantly different from group A and C.^b Change from baseline significantly different from group A.

Discussion

Growth response

The present study shows that raising the GH dose in subsequent years results in a significant, dose-dependent increase of linear growth expressed as HV or HSDS_{CA}. After 4 yr of GH treatment only the higher dose groups B and C differed significantly from the constant-dose group A, in terms of gain in cm, and expressed as the change in HVSDS_{CA} (relative to baseline) and in HSDS_{CA}; however, after 4 yr, group C was no longer different from group B. Although during the course of the study bone maturation proceeded significantly faster than that estimated in untreated girls, there was no significant difference between the treatment groups. Bone maturation was negatively related with baseline CA and positively with the degree of BA retardation. The gain in height outweighed the increase in bone maturation, therefore FH prediction improved markedly, the magnitude being dependent on the method used, but not significantly different between groups. Age, BA RUS, BA retardation, or HSDS_{CA} at start of therapy was not related to the change in HSDS_{CA} over 4 yr in this study group aged 2–11 yr. On the other hand, the gain in height over estimated untreated values as well as the change in PTS_{RUS} after 4 yr of GH treatment were negatively related to prestudy CA, BA RUS, or to the change in bone maturation. A repeated measures model showed that each yearly change in HSDS_{CA} significantly correlated with IGF-I plasma levels.

Dose-response studies

Dose-response relationships in GH-deficient patients have been described earlier (20). De Muinck Keizer-Schrama *et al.* (10) reported in GHD children a significantly higher HVSDS and HSDS_{CA} in 17 transfer patients (previously treated with 12 IU GH/m²/week) on 4 vs. 2 IU GH/m²/day. Preliminary reports in TS indicated that the increase in HV outweighed the increase in bone maturation and therefore FH prediction was more marked and sustained with higher GH dosages (5, 7, 15). However, a comparison with other studies is difficult because of the differences in design and GH dose, entry criteria (e.g. a lower limit for GH provocative testing), age at start of treatment, variables and duration of study reported, and reference populations used. Takano *et al.* (15) investigated two constant GH dosage regimens in prepubertal girls with TS, 0.5 and 1.0 IU/kg/week (comparable with 2 and 4

IU/m²/day). Dose-dependency was shown by the significantly higher mean change in HVSDS (Japanese references) during the first 4 yr in the highest dose group, in which the dose was similar to group A in the present study, compared with the lower dose group. In the fourth year of treatment HV in the highest dose-group was no longer significantly higher compared with the lower dose-group. The same phenomenon might also develop in our study, since after a prolonged period on fixed doses, only the HV in group C remained significantly higher compared with group A in year 4. Nonetheless, this may already have resulted in a substantial difference in height gain. Since the mean change in HVSDS during the fourth year (see Table 2) was well above zero, the girls still exerted catch-up growth. Only 8 girls showed signs of pubertal development at a median age of 13.2 (range 10.9–15.0) yr. There were no significant differences between this group and the prepubertal girls with respect to growth response and bone maturation after 4 yr GH therapy.

Although FH prediction methods all have their inadequacies, it has been shown in a previous report (29) that the mIPH_{RUS} and PTS_{RUS} methods have the lowest mean error compared with the FH actually reached by girls with TS. Furthermore, FH prediction methods should not be used during growth promoting therapy, since they are based on spontaneous growth. However, since mIPH_{RUS} and PTS_{RUS} both include CA, BA (RUS), and height for the estimation of FH, they reflect the influence of GH on growth as well as bone maturation. In the present study both methods showed significant increases in mean FH prediction after 4 yr of GH therapy, without significant group differences. Only a trend towards higher values could be observed in the higher dose-groups (B and C) for both the actual and estimated (FH prediction) cm gained.

Chaussain *et al.* (6) performed a study in TS (CA ranged from 5–15 yr) with a GH dose of 0.7 IU/kg/week (about 3 IU/m²/day). If HV after 6, 12, or 24 months had not doubled, this dose was increased by the same amount (to a maximum of 2.1 IU/kg/week). Fourteen of those 24 girls (58%) and 49% of all girls in the present study were unable to double their HV on 4 IU GH/m²/day after 6 months, and 71% not after 1 yr (data not shown). After 3 yr, 12 out of the 22 girls (55%) were on the maximum GH dose. In agreement with the present study, increasing the GH dose did not lead to an

acceleration of bone maturation, and FH prediction was therefore improved.

GH, IGF-I, and their main binding proteins

In general, spontaneous as well as stimulated (3, 35, 36) GH levels in prepubertal girls with TS have been reported as being near normal (2, 4, 35, 37–39). Despite differences in the assays used, both spontaneous and stimulated GH plasma levels were comparable with those in prepubertal Dutch TS girls in another study (4). In the present study the maximum GH levels after arginine stimulation were similar between groups, but the range was very wide (3–74 mU/L). Fifty-four percent of the girls had a maximum level below 20 mU/L, the cut-off point generally accepted to define GH deficiency. Although the mean BMI-SD score was close to zero in these girls (Table 1), obesity could explain the rather low maximal GH levels in these girls (3, 40, 41), since a negative correlation between stimulated GH levels and baseline BMI-SDS was found. Although representing only a single test, stimulated GH levels were related to the change in HSDS_{CA} after 4 yr GH treatment at the 0.07 level of significance. This is in agreement with a report in normal children (42) and in disagreement with a Japanese report in TS (3).

At baseline, a good correlation between spontaneous and stimulated maximum GH levels was observed in a subgroup of 12 girls of group C, in accordance with a previous report (41). Compared with prepubertal Dutch TS girls in an earlier study (4), and assuming a conversion factor from $\mu\text{g/L}$ to mU/L of 2, maximum GH levels were comparable (25.6 *vs.* 27.6 mU/L), mean 24-h GH levels were rather low (4.3 *vs.* ± 6.0 mU/L), and the mean number of peaks was high (10.2 *vs.* 4.5) in the present study. It has been suggested (4) that an elevated spontaneous pulse frequency pattern might be associated with relatively low IGF-I levels and slow baseline growth and that these girls might benefit most from GH treatment. This seems in line with the present study. Furthermore, the change in HSDS_{CA} after 4 yr GH treatment was negatively related to baseline IGF-I and IGFBP-3 levels (or their SD-scores for CA) or their ratio.

In agreement with earlier short-term findings in GHD patients (20), a clear-cut increase in maximum GH plasma levels and AUC values was observed during GH treatment with increasing dosages. The elimination half-time, however, was similar between the three GH dosage regimens, suggesting that increasing the GH dose results in a higher bio-availability of GH without accumulation. In addition, injection of higher dosages (and volume) did not result in a delayed time to maximum plasma GH levels. Nevertheless, in this small subgroup of girls the change over time of the plasma GH AUC was not significantly related to the corresponding growth response expressed as change in HSDS_{CA} nor to the corresponding change in IGF-I or IGFBP-3 levels or their ratio.

Except for the 30 months time-point in group B, IGF-I levels showed a progressive significant increase during the 4 treatment years, which in part can be explained by the age-dependency of this measurement. At 48 months only

group C still had significantly higher IGF-I levels (adjusted for baseline) than group A ($P = 0.008$). In a report after 3 yr in Japanese girls with TS (43), mean IGF-I levels were statistically higher with 1 IU GH/kg/wk than with 0.5 IU/kg/wk. In the present study the change in IGF-I levels after 4 yr GH therapy was dependent on the dose, the duration of treatment, and the baseline IGF-I level. Thirty-one percent of the girls had plasma IGF-I levels after 4 yr GH of more than P95 for normal girls at the pubertal peak, without significant differences between groups. Only two of these girls were younger than 10 yr. At a lower GH dose than used in group A in the present study (0.68 IU/kg/wk) Ranke *et al.* (44) reported 15% of the TS girls to have IGF-I plasma levels above the pubertal peak after 1 yr. Moreover, baseline IGFBP-3 plasma levels in that study hardly deviated from the normal range, but after 1 yr of GH therapy more than 20% of the girls had IGFBP-3 levels above the pubertal peak. In the present study, determined in the same laboratory, at baseline only 3, and after 30 months 23, girls (35%) had IGFBP-3 levels greater than 5 mg/L (P95 at peak pubertal level), with a similar distribution between the groups. IGFBP-3 was not determined at 42 and 48 months, but a plateau seemed to have been reached after 6 months of therapy, despite age-dependency of this binding protein and a further increase of the GH dose. Baseline log-values of IGF-I *vs.* IGFBP-3 levels showed a significant positive correlation ($r = 0.75$; $P < 0.0001$), but also the change of these values after 30 months from baseline was significantly positively correlated ($r = 0.25$; $P = 0.01$). This is in line with earlier findings in TS (44, 45), and not unexpected, since both proteins are GH dependent. A progressive rise of the IGF-I to IGFBP-3 ratio could be an indicator of the growth response (44). However, after 30 or 48 months of treatment neither was a significant relationship observed between the change in IGF-I to IGFBP-3 ratio and the change in HSDS_{CA}, nor was there a significant difference between groups of the change in this ratio, although a trend was apparent after 30 months: the mean change in IGF-I to IGFBP-3 ratio was 60, 82, and 81, for group A, B, and C. Also, neither IGF-I nor IGFBP-3 levels, nor their ratio were related to the pretreatment HV(SD score). Taken together, there is little evidence to support an explanation of the differences in growth response between the groups by a change in the IGF-I to IGFBP-3 ratio. Nevertheless, a repeated measures model, with the dose-increment steps and duration of treatment as covariants, showed that each change in HSDS_{CA} correlated significantly with IGF-I plasma levels. Thus, free IGF-I might still be a determining factor.

In contrast to an earlier study with an older group of girls with TS (32), but in agreement with another report in TS (46), the decrease in GHBP levels after 6 months treatment was not significant from baseline. This might be because of a large interindividual variation. At baseline there were no differences between groups. Most of the girls had GHBP levels within the normal age range, as shown previously (34). At baseline a linear relationship between GHBP levels and age or stimulated GH levels at start of treatment in the present group of girls with TS was not observed, in contrast to earlier reports in normal children (34). In agreement with the latter

report, however, GHBP levels at baseline correlated positively with BMI-SD score.

In conclusion, a stepwise GH-dosing approach reduced the waning effect of the growth response after 4 yr treatment without undue bone maturation. The increase in FH prediction was not significantly different between treatment groups. Irrespective of the GH dose used, initiation of GH treatment at a younger age is beneficial in terms of cm gained either, at end of study or in terms of predicted FH, but not when expressed as the change in HSDS_{CA} over the study period. The lower the baseline IGF-I and IGFBP-3 plasma levels as well as their ratio, and the higher the change in IGF-I and IGFBP-3 plasma levels, the greater is the change in HSDS_{CA}. The ultimate proof of the effect of the three GH treatment regimens is FH. Therefore, the present treatment protocol will be extended until FH is reached.

Acknowledgments

We gratefully acknowledge the support of Anne van de Wiel in performing this study. Without her experienced physical and mental help this long-term multicenter trial would not have been possible. We thank Wil Hackeng (GH), Gerry Baumann (GHBP), Jaap van Doorn (IGF-I), and Werner Blum (IGFBP-3), and their co-workers for their skillful laboratory assistance. We are indebted to Berthon Rikken for his systems support and to Johan Mouton for his assistance with the GH pharmacokinetics.

References

1. Albertsson-Wikland K, Rosberg S. 1991 Pattern of spontaneous growth hormone secretion in Turner syndrome. In: Ranke MB, Rosenfeld R, eds. *Turner syndrome: Growth promoting therapies*, Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division); 23–28.
2. Wit JM, Massarano AA, Kamp GA, et al. 1992 Growth hormone (GH) secretion in Turner girls as determined by time series analysis. *Acta Endocrinol.* 127:7–12.
3. Tanaka T, Hibi I, Shizume K. 1991 GH secretion capacity in Turner syndrome and its influence on the effect of GH treatment. In: Ranke MB, Rosenfeld R, eds. *Turner syndrome: Growth promoting therapies*, Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division); 41–45.
4. Kamp GA, Kuilboer MM, Wynne HJ, et al. 1993 Slow baseline growth and a good response to growth hormone (GH) therapy are related to elevated spontaneous GH pulse frequency in girls with Turner's syndrome. *J Clin Endocrinol Metab.* 76:1604–1609.
5. Stahnke N, Stubbe P, Attanasio A, Reinhardt D, Partsch CJ, Sippell WG. 1993 GH therapy alone or together with oxandrolone in 212 patients with Turner syndrome (TS): The German experience. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 315–322.
6. Chaussain JL, Gendrel C, Job B, Ducet JP. 1993 Optimizing of the dose of growth hormone in girls with Turner syndrome. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 273–278.
7. Spoudeas HA, Motta RM, Stanhope R, Hindmarsh PC, Brook CGD. 1993 Therapeutic options for promoting growth in the Turner syndrome. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 323–326.
8. Albertsson-Wikland K, Westphal O, Westgren U. 1986 Daily subcutaneous administration of human growth hormone in growth hormone deficient children. *Acta Paediatr Scand.* 75:89–97.
9. Job JC, Chaussain JL, Garnier P, Rolland A, Joab N. 1987 Dose-response relationship in the treatment of hypopituitary children with human growth hormone: a retrospective survey. *Acta Paediatr Scand [Suppl.]*. 337:93–105.
10. De Muinck Keizer-Schrama S, Rikken B, Hokken-Koelega A, Wit JM, Drop S, the Dutch Growth Hormone Working Group. 1994 Comparative effect of two doses of growth hormone for growth hormone deficiency. *Arch Dis Child.* 71:12–18.
11. Rongen-Westerlaken C, Wit JM. 1991 Growth hormone therapy in Turner syndrome: the Dutch experience. In: Ranke MB, Rosenfeld R, eds. *Turner syndrome: Growth promoting therapies*, Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division); 225–229.
12. Rongen-Westerlaken C, Nienhuis HE, Wit JM, et al. 1993 The effect of four years of growth hormone therapy on body stature and proportions in patients with Turner syndrome. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 293–302.
13. Nienhuis HE, Rongen-Westerlaken C, Wit JM, et al. 1993 Results of long-term therapy with growth hormone in two dose regimens in Turner syndrome. *Horm Res. [Suppl.]*. 39:31–36.
14. Frasier SD, Costin G, Lippe BM, Aceto Jr T, Bunger PF. 1981 A dose-response curve for human growth hormone. *J Clin Endocrinol Metab.* 53:1213–1217.
15. Takano K, Shizume K, Hibi I, et al. 1993 Long-term effects of growth hormone on height in Turner syndrome: The result of a 5-year multicentric study in Japan. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 333–338.
16. Rongen-Westerlaken C, Wit JM, de Muinck Keizer-Schrama SMPF, et al. 1992 Growth hormone treatment in Turner syndrome accelerates growth and skeletal maturation. *Eur J Pediatr.* 151:477–481.
17. Rosenfeld RG, the Genentech National Cooperative study group. 1992 Growth hormone therapy in Turner's syndrome: an update on final height. *Acta Paediatr [Suppl.]*. 383:3–6.
18. Gertner JM, Tamborlane WV, Gianfredi SP, Genel M. 1987 Renewed catch-up growth with increased replacement doses of human growth hormone. *J Pediatr.* 110:425–428.
19. Bundak R, Hindmarsh PC, Smith PJ, Brook CGD. 1988 Long-term auxologic effects of human growth hormone. *J Pediatr.* 112:875–879.
20. Jørgensen JOL, Flyvbjerg A, Lauritzen T, Alberti KGMM, Ørskov H, Christiansen JS. 1988 Dose-response studies with biosynthetic human growth hormone (GH) in GH-deficient patients. *J Clin Endocrinol Metab.* 67:36–40.
21. Roede MJ, Van Wieringen JC. 1985 Growth diagrams 1980. Netherlands third nationwide survey. *Tijdschr Soc Gezondh. [Suppl.]*. 63:1–34.
22. Tanner JM, Whitehouse RH. 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child.* 51:170–179.
23. Cameron N. 1978 The methods of auxological anthropometry. In: Falkner F, Tanner JM, eds. *Human Growth, 2, Postnatal Growth*, London: Tindall.
24. Karlberg J, Albertsson-Wikland K, Naeraa RW, Rongen-Westerlaken C, Wit JM. 1993 Reference values for spontaneous growth in Turner girls and its use in estimating treatment effects. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.; 83–92.
25. Rolland-Cachera MF, Sempe M, Guillaud-Bataille M, Patois E, Pequignot-Guggenbuhl F, Fautrad V. 1982 Adiposity indices in children. *Am J Clin Nutr.* 78:178–184.
26. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H. 1983 Assessment of skeletal maturity and prediction of adult height (TW2-method). 2nd ed. London: Academic Press.
27. Van Teunenbroek A, de Waal W, Roks A, et al. 1996 Computer Aided Skeletal Age Scores (CASAS) in healthy children, girls with Turner syndrome, and in children with constitutionally tall stature. *Pediatr Res.* 39:360–367.
28. Lenko HL. 1979 Prediction of adult height with various methods in Finnish children. *Acta Paediatr Scand.* 68:85–92.
29. Van Teunenbroek A, Stijnen T, Otten B, et al. 1996 A regression method including chronological and bone age for predicting final height in Turner syndrome, with a comparison of existing methods. *Acta Paediatr Scand.* 85: 413–420.
30. Hokken-Koelega ACS, Hackeng WHL, Stijnen T, Wit JM, de Muinck Keizer-Schrama SMPF, Drop SLS. 1990 24-Hour plasma GH profiles, urinary GH excretion and plasma IGF-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. *J Clin Endocrinol Metab.* 71:688–695.
31. Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR. 1990 A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. *J Clin Endocrinol Metab.* 70:1292–1298.
32. Van Teunenbroek A, de Muinck Keizer-Schrama SMPF, Stijnen T, et al. 1993 Effect of growth hormone administration frequency on 24-hour growth hormone profiles and levels of other growth related parameters in girls with Turner's syndrome. *Clin Endocrinol.* 39:77–84.
33. Carlsson LMS, Rowland AM, Clark RG, Gesundheit N, Wong WLT. 1991 Ligand-mediated immunofunctional assay for quantitation of growth hormone-binding protein in human blood. *J Clin Endocrinol Metab.* 73:1216–1223.
34. Carlsson LMS, Attie KM, Compton PG, Vitangcol RV, Merimee TJ. 1994 Reduced concentration of serum growth hormone-binding protein in children with idiopathic short stature. *J Clin Endocrinol Metab.* 78:1325–1330.
35. Ranke MB, Blum WF, Haug F, et al. 1987 Growth hormone, somatomedin levels and growth regulation in Turner's syndrome. *Acta Endocrinol (Copenh.)*. 116:305–313.
36. Cappa M, Loche S, Borelli P, et al. 1987 Growth hormone response to growth hormone releasing hormone 1–40 in Turner's syndrome. *Horm Res.* 27:1–6.
37. Ross JL, Long LM, Loriaux DL, Cutler GBJ. 1985 Growth hormone secretory dynamics in Turner syndrome. *J Pediatr.* 106:202–206.
38. Lin TH, Kirkland JL, Kirkland RT. 1988 Growth hormone assessment and short-term treatment with growth hormone in Turner syndrome. *J Pediatr.* 112:919–922.

39. Lanes R, Brito S, Suniaga M, Moncada G, Borges M. 1990 Growth hormone secretion in pubertal age patients with Turner's syndrome. *J Clin Endocrinol Metab.* 71:770-772.
40. Veldhuis JD, Iranmanesh H, Ho KKY, Waters MJ, Johnson ML, Lizarralde G. 1991 Dual effects in pulsatile growth hormone secretion, clearance subserve the hyposomatotropism of obesity in man. *J Clin Endocrinol Metab.* 72:51-59.
41. Frisch H, Häusler G, Blümel P, Schmitt K, Tulzer W, Plöchl E. 1991 Relationship of spontaneous nocturnal GH secretion to GH stimulation and the influence of estrogen pretreatment in Turner syndrome. In: Ranke MB, Rosenfeld R, eds. *Turner syndrome: Growth promoting therapies*, Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division): 35-40.
42. Albertsson-Wikland K. 1986 Growth hormone treatment in short children. *Acta Paediatr Scand.* 325:64-70.
43. Takano K, Shizume K, Hibi I, the members of the committee for the treatment of Turner's syndrome. 1992 Treatment of 46 patients with Turner's syndrome with recombinant human growth hormone (YM-17798) for three years: a multicentre study. *Acta Endocrinol (Copenh).* 126:296-302.
44. Ranke MB, Frisch H, Blum WF, Wollmann H, Bruegmann G. 1993 Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) during treatment with growth hormone in Ullrich-Turner syndrome. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.: 239-244.
45. Ranke MB, Blum WF, Frisch H. 1989 The acid-stable subunit of insulin-like growth factor binding protein in disorders of growth. In: Drop SLS, Hintz RL, eds. *Insulin-like growth factor binding proteins*, Amsterdam: Elsevier Science Publishers B.V.: 103-113.
46. Carlsson LMS, Albertsson-Wikland K. 1993 Growth hormone-binding protein concentration in girls with Turner syndrome: Effects of GH, oxandrolone and ethinyl estradiol. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome*, Amsterdam: Elsevier Science Publishers B.V.: 255-260.